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The Disaster Relief and Emergency Medical Services (DREAMS™) project is a consortium of scientists, medical professionals, and engineers from The Texas A&M University System and the University of Texas Health Science Center at Houston. The goal of DREAMS is to improve the diagnosis and treatment of critically ill or injured soldiers in the field by expediting their access to medical experts at trauma centers of field hospitals.

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INTRODUCTION

The Disaster Relief and Emergency Medical Services (DREAMS™) project has established a consortium of molecular research scientists, emergency medical professionals, and informational sciences engineers from The Texas A&M University System in College Station and the University of Texas Health Science Center at Houston. The primary goal of the DREAMS project is to improve the real-time diagnosis and treatment of critically ill or injured soldiers in the field by expediting the access of "first responders" to medical experts at trauma centers or field hospitals and providing accurate detection of the presence of Chemical and biological Threat Agents. Texas A&M University System research scientists and engineers are working on two components of the DREAMS program:

1. Texas A&M University System (TAMUS) Digital EMS, and
2. Detection and Remediation of Chemical Threat Agents.

Texas A&M University System Digital EMS

Texas A&M Digital EMS is the DREAMS component that allows trauma specialists to treat patients more quickly by providing the "virtual" presence of a physician on the battlefield or at the emergency scene. Digital EMS focuses on integrating existing and "state-of –the-art" developing technologies into the DREAMS Interact ambulance. These include multiple leading-edge telecommunications technologies, especially video processing, wireless communications, and innovate uses of COTS digital systems. The Digital EMS ambulance phase one prototype connects emergency medical personnel on the scene with trauma specialists in distant hospitals. This allows physicians to monitor patients using real-time video and vital signs data from a suite of advanced digital medical monitoring equipment normally only available in a clinic or hospital.

Detection and Remediation of Chemical Threat Agents

The component of DREAMS dedicated to the detection and remediation of chemical and biological threat agents that is critical to the welfare of the soldier in the field and citizens at home. One of the newly emerging concerns regarding military and civilian disaster response scenarios is the involvement of chemical or biological weapons of mass destruction, which might range from noxious chemical fumigants to neurotoxic, chemical warfare agents, or from infective biological spores and viruses or their biologically-active toxins. Texas A&M scientists are developing genetically engineered enzymes that recognize and decontaminate a host of chemical and biological threat agents, and methods to integrate these new materials into detection and decontamination systems.

BODY

The Texas A&M University System Digital EMS

Research Accomplishments

Task 1: Development of Site Specific Test Plans

The Texas A&M University System (TAMUS) continued to meet periodically with the Liberty County EMS to review operation and testing protocols for deployment. Coordination with Liberty County EMS for the first ambulance fielding was finalized. Development of test protocol and operation protocols support documents continued throughout the period.

Meetings were held with Liberty County Hospital to establish requirements for their participation as a rural hospital deployment site. These discussions were greatly protracted by requirements to satisfy their owners and insurers. Final agreements are expected in early 2004. During the year several communications tests were performed in College Station and Liberty County to determine additional testing parameters required during field testing.

Figure 1: Liberty County Hospital ER Room

Multiple engineering tests have been completed in Liberty County with LCEMS paramedic participation. Functionality test plans and reports are prepared for each test run. August results indicated a severe limitation of cell phone coverage to major thoroughfares in Liberty County. Based on the results we are planning a hybrid communications approach utilizing two or more cell phone providers. This would enable a broader coverage area and enable us to

use more cellular devices while reducing our communication traffic collisions. The September tests verified that our communications system now successfully connects and re-connects as the ambulance leaves and re-enters cell phone coverage zones.

Task 2: Digital Ambulance Systems Evaluation, Development and Integration

TAMUS personnel continue to improve the hardware and software components specified by the digital ambulance system functional requirements.

Intelligent Communication Manager (ICM) development has focused on the increased reliability of fragmented packets flowing through the ICM gateway. A Digital EMS fragmentation and buffering system has been implemented to allow for packets that are longer than the Ethernet defined maximum Transfer Unit (MTU) to be fragmented by a local procedure that attaches an additional ICM header used for buffering incoming packet segments. As packets are received on the physician ICM side, the header is extracted and used to buffer and reconstruct complete packets before being sent out to the destination client. Packet fragments that do not meet a predefined timeout are dropped from the system buffer so as not to increase the delay of subsequent fragments belonging to other incoming packets. The previous ICM utilized 8 dedicated cell phones and modems thereby enabling the ICM to have direct control over the sending and receiving of data. The ICM has been re-engineered to accommodate a new communications topology in which the 8 new 3G cell phone data is routed onto the Sprint network and arrives at the physicians workstation on a single Ethernet interface. These ICM revisions were field tested in Liberty County.

Meetings continue between A&M team members and Liberty County EMS. Some modifications have been made based upon Liberty County EMS requirements. The Liberty County ambulance was outfitted with a complete DREAMS hardware suite. The new equipment multiplexes 4 cell phones to one antenna thereby reducing the openings into the ambulance box. During a site visit by Liberty County, it was recommended that two barcode scanners be installed to allow accessibility from either side of the patient. The scanners were located so the beam spread is absorbed and will not interfere with line of sight. A signature capture pad, an encrypted wireless headset and a thermal printer were integrated into the system and installed in the ambulance. Software was re-engineered during this year to accommodate Liberty County run record forms and a new user interface design. The team integrated the vital signs monitor, bar code scanner and card reader technologies into the new forms.

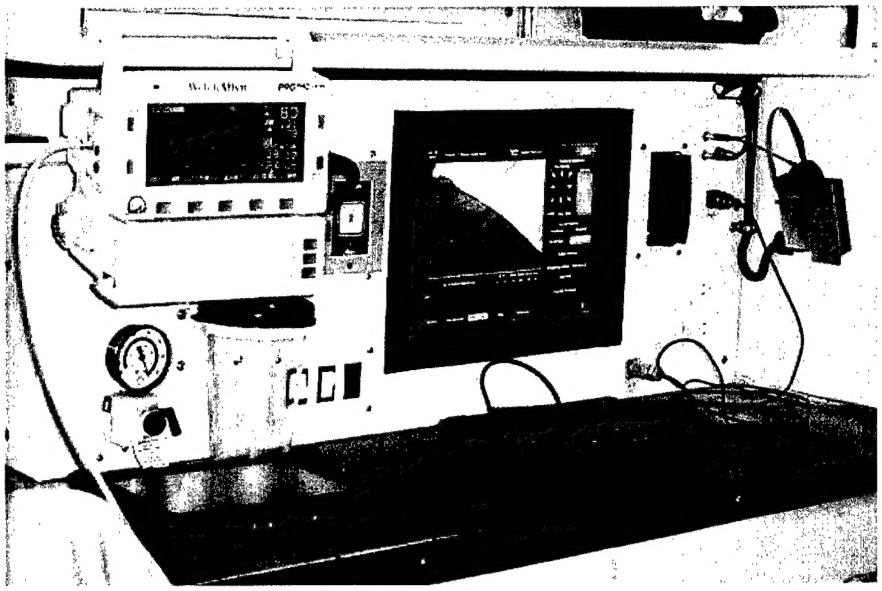

Figure 2: Hardware integrated into the Liberty County ambulance

Software was delivered to two independent groups for evaluation: SHIS for usability evaluation and the Network Lab group for a thorough functional evaluation. Engineers designed and implemented the case for the phones consisting of: a single dc power supply, mobile telephone package of 8 cell phones, variable adjusting amplifier and an antenna multiplexer. As a result of engineering testing, it was determined that there was a need for an auto adjusting capability where the server adjusts the video frame rate based upon the amount of available bandwidth. This capability is currently in development. Periodic support is continually provided to Liberty County EMS for their interactions with county officials, and other community leaders.

Task 3: Hospital-Side Systems Evaluation, Development and Integration with Digital Ambulance

In February, members from UTHSC and A&M made a site visit to the Liberty County Hospital. This meeting was to inspect the communications infrastructure for connection to Herman Hospital and to assess the space requirements for a physicians' workstation. Discussions were held about placing a DTS system in their emergency room and evaluating as part of the relationship. The team is awaiting responses from Liberty County hospital as to suitability for DREAMS test and evaluation site. Their management company is investigating insurance and other liability issues with the underwriters. They are in the process of negotiating a new relationship with Herman Hospital to cover the medical and clinical aspects of the DREAMS partnership.

Physician workstation hardware has been placed on order for support of the Liberty County EMS and installation of equipment at both the LCEMS offices and at Herman Hospital.

Task 4: Develop Communications Infrastructure to Support Hospital-Side and Remote-Site Communications Systems

The previously designed radio and satellite equipment required to support communications to and from remote ambulances and DTS test sites has been partially implemented during this period. This equipment supports Ku band satellite digital data and video and 802.11b wireless connections. A DREAMS engineering team traveled to NRL to coordinate the development and 4th phase testing of the mobile satellite terminal. The ground station for installation on the IBT building in the Texas Medical Center was prepared for installation. However, shipping damage to the main satellite dish was discovered and the installation was rescheduled for October. Testing is now scheduled for late October/early November with a final acceptance in December. This testing will also include certification testing with the Dreams remote, ambulance based antenna system.

An 802.11b wireless bridge was installed in Liberty County ambulance to facilitate wireless communications with an administrative station at the Liberty County main office. Planning is underway on the requirements of this administrative system for receiving and archiving of digital run records. Investigations on new third generation (3G) cell phone technology continue. Several new generation cell phones were tested for suitability. Operational decisions by the telecomm providers continue to limit the actual performance improvements the new technology is able to deliver versus the technologies' potential. Tests will continue to be conducted to determine actual results. IMMARSAT satellite service support was awarded to GCS and further testing will continue.

Engineers designed and implemented a portable case for the cell phones consisting of: a single dc power supply, 8 cell phones, variable dynamically adjusting amplifier and dual antennae multiplexers. The existing ICM utilized 8 dedicated cell phones and modems thereby enabling the ICM to have direct control over the sending and receiving of data. The ICM has been re-engineered to accommodate a new communications topology in which the 8 new 3G cell phone data is routed onto the Sprint network and arrives at the physicians workstation on a single Ethernet interface.

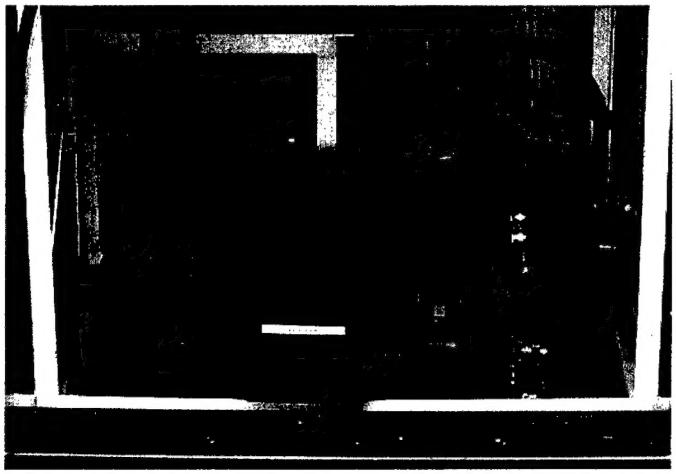

Figure 3: Portable case for cell phones

Two DREAMS staff members were trained in the use of the Marconi AN/GRC 245 military radio system. This system is too large and cumbersome to be a viable solution for the current DREAMS HMMWV demonstration requirements. However, the evaluation of the Marconi AN/GRC 245 military radio system continued and was performed to a slightly better level of detail relating to the technical characteristics of the radio (as opposed to environmental issues such as size weight and power.). Based on this evaluation, even if size, weight, power and price issues could be resolved, other alternative systems are likely to meet the project requirements and consequently, on a technical basis, the AN/GRC-245 is still not a viable solution. We are acquiring Motorola 5000 series radios for evaluation in late 2003. These are the Air Force radio of choice for the early spring demonstration.

Task 5: Integrate New Technologies into the Existing System Architecture; Enhance System Functionality and Support Upgrades

The new ambulance was received and required DREAMS modifications to the vehicle were completed. All required DREAMS computing and communications equipment was integrated in preparation for testing and actual deployment in Liberty Co, TX.

Work was done to update cell phone antenna arrays and minimize signal loss due to poor or unwanted electronic coupling. The 802.11b system was updated with newer model bridges to provide more control, enhanced functionality, adherence to current standards, and improve performance. An improved, ruggedized computer mounting system was designed and installed in the Liberty County vehicle. The UPS system was upgraded which reduced weight

and size requirements as well as allowing remote control and monitoring of the UPS. A new version of the ICM technology was installed and tested.
Previous work continued on improving and modeling the performance of the Sprint 3rd generation cell phones. During this reporting period, the cell phone communication system was converted to use PCMCIA cell phone cards such as those from Sierra Wireless. These cards have been incorporated into a PC104+ based computer system that is miniaturized and ruggedized with no moving parts. Multiple cards operating on each of 2 separate networks provides redundancy for adequate bandwidth and infrastructure redundancy to insure network coverage by using multiple network systems. Testing is continuing but to date has shown that this system should be very effective. The team purchased, installed and integrated a wireless tablet pc in the driver's cab of the Liberty County ambulance. This mobile form factor enables data collection outside the vehicle that is immediately sent to the physician.
Paramedic and physician interfaces were moved from the initial prototype stage into full-scale operational versions. A new version of the Intelligent Communication Manager (ICM) software was installed and tested.
Work continued on the integration of a full SQL compliant database system into the ambulance architecture. This allows full tracking and querying of patient information, in addition to facilitating better management of patient records when they are unloaded from the vehicle to the main EMS office. The software database connection is engineered such that database table design established during run time is generalized. The advantage is that the underlying database design is driven by the top-level user interface and not by any particular form or form structures.

Task 6: Initiate Field Testing of Digital EMS System(s)

Staged runs of the Digital EMS system were originally slated to begin in Spring 2002, but, due to the limitations imposed by 10 USC 980, DREAMS project staff has had to postpone all testing involving any patients whether real or healthy normal volunteers.

DREAMS researchers have continued in regular contact with TATRC and USAMRMC personnel in order to develop a strategy for gaining an exception. Phase zero testing using DREAMS staff was performed in the 4th qtr. Phase one permission is expected in late December or early January. Meanwhile, researchers continue to plan and develop systems for use in the field-testing of the system.

Plans were expanded to include field-testing in Brazos and Webb Counties. The new ambulances to be delivered in early 2004 will serve these areas. Engineering field tests in College Station focused on assessing the viability of the high throughput cell phone technology. Initial results are very promising

although Sprint coverage is restricted to major thoroughfares in Brazos County.

Field-testing continued in Liberty County focusing on assessing paramedic usability and communications viability of the high throughput cell phone technology. The team obtained feedback from paramedics for system modifications. Results are very promising although Sprint coverage is restricted to major thoroughfares in Liberty County also. Testing of a hybrid communications approach utilizing both Sprint and Verizon cellular data modems is planned for early 2004.

Task 7: Design and Develop Initial Prototype of Digital EM System for Military Use

Research personnel work with TATRC personnel to coordinate a concept development team in order to develop the next generation military ambulance through a continual series of meetings. Meetings were held January 2003 with subsequent meetings held in the Summer.

Multiple enhancements or updates were installed and tested in the HMMWV during this period. The HMMWV was originally outfitted with 1RU rack mount computer hardware. The new computer system has been redesigned to one/third the volume and about one/third the weight. All computer and communications equipment is DC and consume significantly less power. A prototype is under construction and will begin testing in November. Two revisions of the shock/vibration isolated computer rack for the HMMWV were tested and redesigned. Additional monitoring cameras, video recording systems, updated computer systems, modified power distribution, updated cell antenna array, updated GPS system were installed. The HMMWV was not shipped to Auragen for AC power generator installation due to fuel system problems. A decision was made to delay Auragen installation until after the Marine tests and subsequent evolution has eliminated the requirement for the additional AC power at this time. A 1 to 2-kva power inverter was installed instead to provide the AC power requirements of the testing

A new version of the ICM technology was installed and tested. The new DREAMS software release including GUI was installed and tested on the HMMWV onboard computers. The SimMan simulator was integrated into the ambulance for mobile training activities.

During this reporting period, we have been investigating new camera technology. As a result of the CIBRF demonstration, the need for better cameras has become very apparent. Consequently, we are searching for higher resolution, lower light systems with more efficient digital interfaces. Several candidates have been selected and samples are being evaluated. The

ruggedized, waterproof camera system has evolved and bench testing has completed. Circuit boards had to be redesigned to allow additional control circuits.

A new video control system is being designed using PC104+ components. This is not expected to test until early 2004.

Task 8: Participate in Military Tests, Concept Evaluations and Exercises

DREAMS members participated in the CBIRF Technology Demonstration Day on 15 November 2002 at Indian Head Naval Weapons Station in Maryland. The Deployable Telemedicine System (DTS), physicians workstation and the Medi-cam were demonstrated.

The HMMWV DREAMS prototype participated in several test exercises during 18-20 June 2003 with the CIBRF team at Indian Head and Stump Neck, both of which are training and testing locations for Naval Surface Warfare Systems. These demonstrations were to acquaint the CIBRF team with the capabilities of the DREAMS HMMWV variant and the DTS field deployable system, to demonstrate the DREAMS satellite terminal, to demonstrate the use of video as a tool for the CIBRF extraction teams, and to elicit input from the CIBRF participants for future DREAMS developments. A demonstration/test for the Air Force is tentatively scheduled for 1st Qtr 2004 at Ft. Dietrich.

Task 9: Evaluate and pursue opportunities to develop and implement the Digital EMS system in disaster response scenarios

Development has continued on the new version of DTS that conforms to two man carry limitations, has expanded power options and occupies less volume and weight. The prototype is expected in Spring 2004.

Digital EMS was approached as a potential participant in a 3-way demonstration test bed for remote, satellite based communications offload in case of massive local failure of telecommunications due to natural disaster or Homeland Defense issues. Further discussions have been scheduled for first Qtr 2004. The participants under discussion are Chicago, Houston, and Indianapolis.

Small, man-wearable video cameras and transmitters, such as the MediCams (from TATRC), have use in military, terrorist, and civilian disaster scenarios. Due to limitations with the MediCam units, we have spent some effort to understand the source of the MediCam problems, and have concluded that the antenna reflectors are likely to have been damaged (probably during shipping) and that this has contributed to the lack of effectiveness of the current

MediCams. Triggered by these limitations, we developed a small, light, belt-worn video transmitter that could be connected to a standard HMMWV DREAMS camera. This unit fulfilled the CIBRF requirement to be completely waterproof and be decontaminated easily and quickly. The demonstrations with CIBRF have highlighted the requirement for more powerful video transmission capabilities. The iron and steel filled rubble pile used by CIBRF as a training site critically demonstrated the requirement for more signal penetration power and extended reach for the radio link. The DREAMS group continued investigating more powerful, less intrusive camera solutions for future demonstrations. We have also been developing the conceptual framework and system design for repeater modules to support the CIBRF application. This will provide ability to extend transmission range by using small, disaster worker deployed RF repeaters. We have been studying high performance patch antennas. These directional antennas have shown great promise so far to extending the range of video systems. The SimMan simulator was integrated into the ambulance for mobile training activities.

Task 10: Work with University of Texas Health Science Center to Enhance Current Technologies within the Digital EMS Vehicle and Associated Hospital Systems

In previous periods, TAMUS developed a series of use cases for the digital ambulance to use in refining system requirements, and in testing the functionality of the systems. Additional cases were defined and the previous ones reviewed and updated.

Evaluation of new medical technology and portable equipment continued through this year. Focus was on convincing manufacturers to allow remote software access and control of vital signs monitoring so that additional monitors can be qualified. The UTHSC evaluated the new wireless version of the Welch-Allyn Propaq Encore. This technology fits well with the DREAMS mission and will be further pursued.

Task 11: Enhance the Existing Digital EMS System to Accommodate Additional Functionality

A significant portion of the Digital EMS code has been rewritten for speed, size, and functionality. More functions have been moved to additional specialized hardware that allows significant improvements in speed. Remote site software has been re-engineered to improve performance characteristics so that within seconds after a value is entered that data is sent to the physician. This ensures that the physician now has the most accurate information continually. A database synchronization mechanism was developed to ensure immediate and accurate data updates.

Significant hardware redesign and upgrade has greatly reduces size, weight, and power requirements for computing and communications systems. Research continued to further enhance the quality and extend the reach of portable video delivered from the EMT to the ambulance to the physician. A rugged, waterproof wide-angle lens camera was developed. This camera has been adapted for use wired or wireless.

Task 12: Integrate New Technologies for Inclusion in the Digital EMS Vehicle to Support Additional Medical Functionality for Trauma Care at Remote and Hospital Sites

The telestration system was completed and is ready for deployment when required. As part of this the physician workstation has been augmented to include a digital pen and tablet device enabling the physician to annotate the video image and broadcast to the remote site.
A touch screen tablet pc was installed to serve as the driver's station. This smaller, mobile form factor enables data collection outside the vehicle that can be immediately sent to the physician.

TAMUS personnel continue to coordinate with UTHSC personnel to test and evaluate capabilities that will identify additional technologies and functionality for integration into the system. Communication with IMPACT Instrumentation, Inc. was begun to investigate integrating their new network based portable ventilator. This device has been requested by the U.S. Air Force for use during their test scheduled for 1st quarter 2004.

Task 13: Integrate new US Army, NASA and DARPA Technologies Such as Medic-CAM and WARP into the Existing System to Enhance System Functionality

Significant work was done during this year to tune, repair and stabilize the existing Medi-Cams. These systems were specifically requested by the Navy for the tests with CBIRF. Each unit had one or more problems including broken connections, out of spec cameras, bad connectors, bad or inappropriate filters or they required re-routing of cables or re-tuning to separate the channel spacing for noise minimization. These units were integrated into the HMMWV video equipment for the demo. These systems may also have had damaged antennas

Task 14: Enhance Existing Infrastructure for Supporting a Network of Multiple Digital EMS Vehicles and Hospital Systems in an Integrated Environment

TAMU personnel worked with UTHSC to revise the design for the Ku band up/down satellite terminal located at the IBT.

Work was done on streamlining the data messaging system to allow for easier information exchange between the ambulance and hospital clients/servers. The new ambulance was outfitted with updated 802.11b technology to allow for high bandwidth communication between arriving vehicles and the hospital network when at close range to the hospital. In later phases this system will be used for connecting to in-fill networks located at strategic points within Liberty Co. to allow for higher bandwidth communications when the attending physician requires full motion video.

Engineers are investigating technologies to enhance the physicians workstation ability to receive high bandwidth data from multiple remote sources. These include fiber optic data connection and ultra small aperture satellite downlink.

Task 15: Develop and Test a Prototype Digital EMS Vehicle in Diverse Urban and Rural Technologies for Evaluation and Performance Analysis of Integrated Digital Technologies

TAMUS personnel continued throughout this year to work toward the Liberty County deployment. After the vehicle delivery, the DREAMS enhancements were added and local engineering testing was performed in College Station. Continued delays in receiving the 10 USC 980 permission limited our ability to move out of the engineering test phase. During this phase additional modifications to the vehicle, communications hardware and software were made as evolution continued.

The Liberty county EMS requested several changes and these were added to better accommodate their operations. Field engineering tests were performed throughout the 4th qtr. Initial communications testing was performed in Brazos County in rural and urban areas. Tests identified potential problems in repeated entry and departure from cell phone coverage zones (cells). Investigations into these issues indicated phone hardware deficiencies.

Communications testing has re-commenced in Liberty County, inside and outside the city limits. Tests produced successful results and positive paramedic feedback. Engineers are planning urban and rural testing of a hybrid communications approach utilizing Sprint and Verizon cellular data modems. New design specifications and modifications for the 14' ambulance boxes have been released to Frazer, Inc., the ambulance manufacturer, and construction has begun. These designs include the computer systems and support equipment being built into the ambulance structure instead of using space traditionally given to the paramedics.

Task 16: Develop Methodologies for Using New Local, State and National Network Infrastructures for Providing the Digital EMS Vehicle with High Speed Terrestrial Connectivity to the Hospital Node

Digital EMS was approached in late 2002 as a potential participant in a 3-way demonstration test for remote, satellite based communications offload in case of massive local failure of telecommunications due to natural disaster or Homeland Defense issues. The primary idea is to use a DREAMS like mobile uplink to re-route traffic in the disaster area via satellite to an unaffected part of the country where the network traffic is put onto the public infrastructure. The DREAMS terminal at the IBT is ideal for this use. The city of Chicago has proposed to copy the DREAMS design for their static terminal. Additional discussions were held in early 2003 but the project was put on hold awaiting decisions by the Chicago terminal group. The participants in the discussion are the city of Chicago, DREAMS in Houston, and the city of Indianapolis. New developments are expected in early 2004 as the Chicago satellite terminal comes on line.

Communications tests identified potential problems in repeated entry and departure from cell phone coverage zones (cells). Investigations into these issues indicated phone hardware deficiencies. New generation Sprint cellular telephones and PC CardBus cellular data modems were evaluated for inclusion in future versions of the hardware. It was decided to stop integration of cell phones and focus on new generation modems. These devices increase the data transfer speed as well as reduce weight, power and space requirements. The decision was made to incorporate the PC CardBus devices into the ambulance and HMMWV by developing a dedicated modem controlling PC 104 rugged computer system.

Task 17: Publish Findings and Results in Appropriate Conference Proceedings and Journals and Demonstrate Capabilities of the Digital EMS Ambulance

The Digital EMS System was demonstrated and/or the project was presented at the following meetings and conferences:

- Oct 2002 – Trinity Valley Conference in Liberty County, Texas
- Nov 2002 – Tele-Trauma Conference in Vermont
- Nov 2002 – Texas EMS conference in Austin, Texas
- 15 Nov 2002 – CBIRF Tech Day Demonstration at Naval Surface Warfare Center in Indian Head, MD
- 8 Jan 2003 – DREAMS Product Line Review, College Station, Texas
- 20 Feb 2003 – Liberty County Hospital presentation

- 14 May 2003 - Demonstration for Future Combat Systems meeting held in College Station
- 18-20 June 2003 – HMMWV DREAMS variant was demonstrated to CBIRF on a training exercise at the Naval Surface Warfare Center in Indian Head, MD
- 2 September 2003– Presentation to Liberty County Commissioner's Court
- 22-25 September 2003 - National EMS Conference, Las Vegas, NV

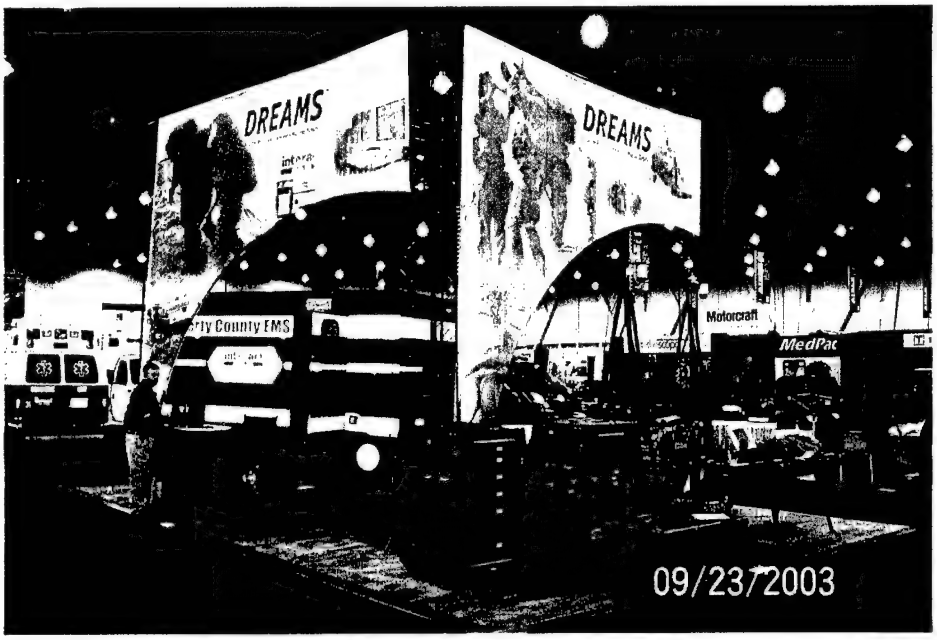

Figure 4: Demonstratrion at National EMS, Las Vegas, Nevada

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

Research Accomplishments

Task 1: Determination of the Catalytic Limits for the Existing Organophosphate (OP) Hydrolases

Detailed biochemical studies have been performed on some of the most thoroughly studied mutations of OP hydrolase (phosphotriesterase; OP

anhydrolase). Selected genetic constructions have been analyzed with actual CW agents and demonstrate significantly enhanced catalytic activity with Kcat values for VX that approximate 40 s-1 and for VR that exceed 200 s-1 (in collaboration with Dr. Douglas Cerasoli at USAMRICD). The effectiveness of various enzymes against actual chemical warfare agents has been determined through a collaboration with Dr. Douglas Cerasoli at USAMRICD, Edgewood, Maryland. There is a slight increase in binding association in some of the enzymes since the K_M in some cases has decreased. The turnover values of these new enzymes are adequate for the development of enzyme-based destruction of VX and other V-type agents. There are various trade-offs in these genetic reconstructions in that the enhancement of catalytic turn-over rates for one substrate may be accompanied by a decrease in hydrolytic rates against other substrates.

Co^{2+} enzyme

	Km(mM)	Kcat (sec^{-1})	Kcat/Km ($M^{-1}sec^{-1}$)
WT VX	2.0	5.6	2778
WT VR	16.3	43.4	2672
RL VX	1.7	27.0	15594
RL VR	2.0	214.1	104913
WT d-S	1.6	2.5	1525
RL d-S	3.1	82.0	26000

"WT VX" = Wild type OPH with VX)
"RL VR" = Mutant OPH with VR)
"WT d-S" = Wild type OPH with demeton-S)

Collaborative studies with the French Army and Dr. C. Stephen McDaniel ("Reactive Surfaces Ltd.") (Austin, Texas) was held in Cazaux, France in September 2002 (NATO-WG31, Project Director, Dr. Joseph DeFrank; (SBCCOM, U.S. Army, Edgewood, Maryland) demonstrated the ability of painted reactive surfaces to detoxify Soman (Reactive Surface Ltd.)

Task 2: Investigation of Mutations of Individual Residues and Creation of Rational Combinations

We have completed formation of several single site libraries within the active site of phosphotriesterase. The total number of mutants that have been selected is approximately 240. This library is being screened for improvements in the hydrolysis of a number of chiral analogs of the nerve agents GB, GD, and VX. Thus far we have identified at least two mutants that are an order magnitude faster than the wild type enzyme for the hydrolysis of our VX analog. We are in the process of characterizing these mutations in further detail. This approach looks very promising.

We have selected 67 random mutations that are being screened for enhanced stability in the dimer under denaturing conditions due to elevated heat or higher chemical concentrations.

Task 3: Investigation of Combinatorial Mutagenesis of Amino Acids Affecting the Active Site of the Enzyme

We have identified a promising combinatorial mutant involving three amino acids (H254/H257/L303) surrounding the active site region through our combinatorial libraries that are enhanced for the hydrolysis of chiral GD analogues. This mutant is being characterized to establish the rate enhancement for the hydrolysis of Soman relative to the wild type enzyme.

New combinations of mutations have been created by protein engineering which is designed to create a balance between the stability of the enzyme and a more active catalytic center. Several interesting combinations of three different substitutions have been created and evaluated, but it is premature to provide detailed characterization. The general strategy has been to change the hydrophobic nature of some of the amino acids near the active site of the enzyme in order to increase the affinity of the enzyme for specific chemical characteristics. The evaluation of these new enzymes has begun. These new enzymes were designed to increase the hydrophobicity of the active site ("AS ↑hydrophobicity"), alter the mechanical flexibility in the active site ("AS mechanics alteration"), stabilize the overall structure, remove the tryptophan residues to evaluate potential for utilizing fluorescence probes for stability and substrate binding analysis ("reporter elimination"), separate dimer into catalytically active monomers ("Interface Mutants") , introduce stabilizing covalent cross-linkage ("Thiol Bond Formation"), and a variety of independent mutational changes to pursue various structural interests.)

Task 4: Introduce the Best Currently Existing Enzymes into Existing Biosensor Detectors.

A multi-component, sensitive, optical biosensor has been produced by forming a conjugate of the recognition enzyme OPH with fluorophore reporter elements CNF). The conjugate has been immobilized on fiber optic wave guide of portable fluorescent detector (Analyte 2000). This is the first successful demonstration of paraoxon detection by fiber optic system; the base is the commercially available Analyte 2000. The OPH-CNF-biotin conjugate was immobilized on the plastic wave guide of fiber optic detection system (Analyte 2000) via avidin –biotin chemistry and shown to be able to report OP hydrolysis activity.

There has been a continued development of portable fluorescence-based sensors for the direct detection of OP neurotoxins. Recently we have described a new biosensor for the direct detection of organophosphorus neurotoxins that utilizes the formation of two protons in each hydrolytic turnover through a reaction in which P-O, P-F, or P-S bonds are cleaved. The linear detection range for paraoxon spanned a concentration range of 0.25 - 250 ppm (0.001 mM - 1.0 mM). The sensitivity of this biosensor was limited in the first order by the limitations of potentiometric method of detection. Higher sensitivity may be achieved using fluorescence spectroscopy to detect local pH changes in the vicinity of enzyme active side. Several pH-sensitive fluorophores with different excitation wavelength are available.

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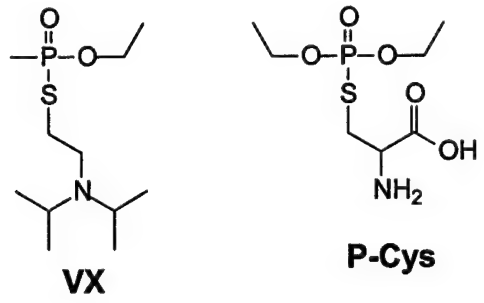
Task 5: Selection and Purification of Mutants with Specifically Desired Catalytic Characteristics

We have concentrated our efforts on the construction of a bacterial selection method for the identification of mutants of phosphotriesterase that are better for the hydrolysis of the P-S bond that is found in VX. Our strategy has been to synthesize an analog of VX that has a cysteine leaving group as shown below. Transformation of the cysteine-auxotrophic cells with a plasmid containing the gene for the wild type phosphotriesterase enables the cells to grow slowly in the presence of the VX-analog. We interpret these results to indicate that the phosphotriesterase that is expressed in the pariplasmic space is hydrolyzing the VX-analog resulting in the production of cysteine. The released cysteine enables the cells to grow. We have also demonstrated that transformation of these same cells with a mutant of phosphotriesterase that is known to hydrolyze the VX-analog with a higher turnover number enables the cells to grow faster and to a higher cell density. These are quite promising results since we will be able to use this selection procedure to identify additional mutations that are now optimized for the cleavage of the P-S bond.

We are currently constructing a large library of mutant plasmids using error-prone PCR.



Task 6: Introduction of the New Genetically Engineered Proteins into New Biological/Chemical Sensors

We have purified a new enzyme from E. coli that has the ability to catalyze the hydrolysis of organophosphate nerve agents. This enzyme will hydrolyze the insecticide paraoxon and cleave the P-F bond in diisopropylfluorophosphate (DFP). The enzyme has been crystallized and we are working with other collaborators to establish the three dimensional structure of the enzyme.

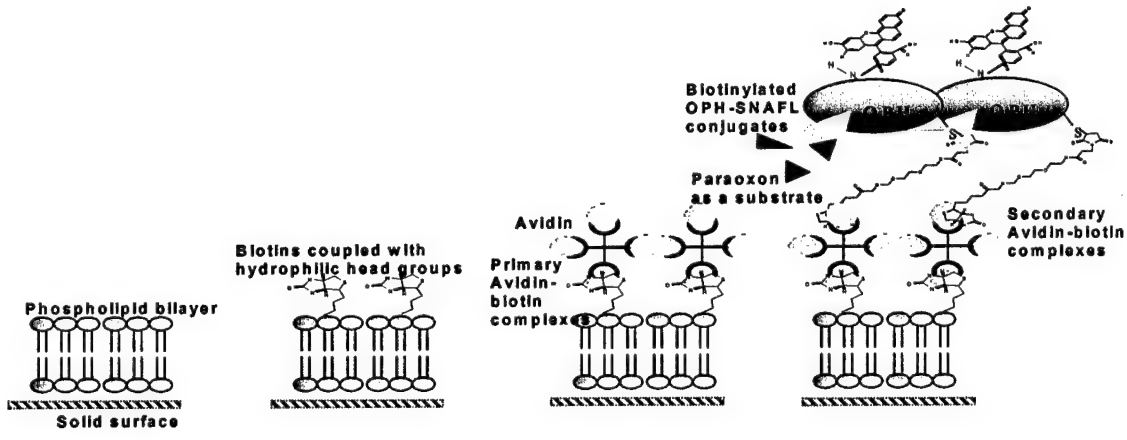


Fig. 1. Conjunction chemistry for OPH-CNF immobilization.

We have begun to prepare surface adhesion complexes that involve OPH in conjugation with biotin as a binding element in a well-controlled biotin/avidin immobilization technique. In addition, a fluorophore dye SNAFL or CNF will

serve as a pH-sensitive reporter (Fig. 1). Such three-particle conjugates (OPH-biotin-fluorescence dye) may be easily immobilized on surfaces such as glass slides, fiber optics or plastic wave guides to create optical biosensor for direct and fast detection of CW agents or organophosphate pesticides. We have begun to develop protocols for biotinylation of wild type OPH and several genetically modified OPH (RL, RS, etc.) and prepared initial three-particle conjugates which include OPH-biotin-SNAFL (Conjugate1) and OPH-biotin-CNF (Conjugate2) with a high concentration of dye.

Task 7: Introduction of the Genetically Designed Enzymes into Decontamination Applications - Collaborations with Industry

1. LynnTech (College Station) "A Cotton Towelette for Nerve Agent Decontamination". DOD SBIR, Phase II -OSD9-033. The development of a self-decontaminating surgical towelette has completed laboratory development and animal studies have just been initiated.

2. LynnTech Inc. (College Station) "Activated Organophosphate Hydrolase for Coupling to Human Skin" has been approved for DOD.STTR Status. This project will develop skin protectant barriers with active decontamination enzyme-based catalysis.

3. New Horizons Diagnostics (Columbia, MD). Multiple Enzyme-based CW Detectors utilizing Butyrl-cholinesterase:: organo- phosphate hydrolase:: choline oxidase, and perhaps peroxidase are being developed as a discriminating biosensor that can discriminate between various classes and subclasses of chemical neurotoxins. (Supported in part by IPP-DOE, Collaboration PNNL!)

Task 8: Development of New Enzyme Biocatalysts for Detection and Destruction of Chemical Warfare

To date we have twelve completely cloned opaA homologs; four of these are novel, six are consistent with published genome sequence variants. Of the four novel ones, three have significant activity against DFP, GA, GB and weak activity against GD. (These have only been tested as cell-free extracts at USAMRIICD). Of the four previously published sequences, two have DFP activity. The remaining three bacterial sequences have sequence homology, but little or no DFPase activity and were cloned for comparison purposes. We have ordered other strains for which the published sequence is known. Since the sequences are known, it should be a quick clone once it arrives. This would make a total of eleven new opaA genes to study.

We have begun to characterize an organophosphate acid anhydrase from E. coli. The enzyme will hydrolyze the entire series of dialkyl p-nitrophenyl

phosphate analogs that was developed for PTE. The enzyme greatly prefers a methyl group at one of the alkyl positions. The preferred stereochemistry is identical to that observed with PTE for these same compounds.

New gene discovery with three different classes of OP neurotoxin degrading enzymes (OPAA, PON, and OPH) has produced the first novel enzymes in several years. The human serum detoxifying enzymes have been genetically cloned and sequenced: they are being over-expressed prior to detailed substrate capacity and initiating genetic engineering studies.

Task 9: Development of a General Approach for High Throughput Screening of Mutant Enzymes for Remediation of Chemical and Biological Agents Using Arrays of Living Cells

A four-step, soft lithographic process based on microcontact printing (μCP) of organic monolayers, hyperbranched polymer grafting, and subsequent polymer functionalization, has resulted in polymer/*n*-alkanethiol patterns that direct the seeding of bacterial cells. The functional units on these surfaces are three-dimensional bacteria "corrals" that are as small as 12 μm square. The corrals have hydrophobic, methyl-terminated *n*-alkanethiol bottoms, which promote bacterial adhesion, and walls consisting of hydrophilic poly(acrylic acid)/poly(ethylene glycol) layered nanocomposites that inhibit adhesion.

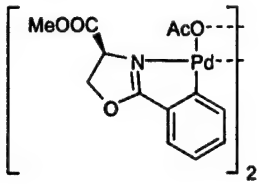
Cell viability studies indicate that cells remain viable on the patterned surfaces. Large corrals (63 μm square) contain 18±5 bacteria and smaller corrals (12 μm square) contain 2±1 bacteria. Bacteria reside within corrals with a reliability of 92±8%; the remaining cells reside on walls between corrals. Applications to bioarrays for high-throughput screening and biosensors are envisioned.

Task 10: Development of Biosensing Systems for Discriminating Between Different Classes of Neurotoxins Based on Coupled AchE-OPH Enzyme Monitors

This task has been completed and the technology transferred to commercial development in collaboration with Pacific Northwest National Laboratory (Dr. Evgenia Rainina. (PNNL-IPP-DOE). There is an on-going collaboration with Dr. Genia Rainina (DOE-IPP Program Manager), Dr. David Trudell (New Horizons Diagnostics, Inc., Columbia, MD), and Dr. Ilya Kurochen (Moscow State University) to develop a third-generation biosensor that is capable of discriminating various types of chemical neurotoxins. Four of the second-generation biosensors are being tested by Dr. DuPont Durst (SBCCOM, Edgewood, MD). We are involved only as periodic consultants on the project.

Task 11: Investigation of the Application of Polynuclear Metal Complexes for the Detection and Destruction of Chemical Warfare Agents.

As part of our efforts on the hydrolysis of organophosphorus nerve agents, we have prepared several palladium complexes bearing hydrophilic chiral oxazoline ligands. An example is provided in Scheme 1. The resulting dinuclear palladium complex has been characterized by NMR as well as by X-ray crystallography. We have started to evaluate the activity of this catalyst in the hydrolysis of parathion and we found that at pH 8, this complex is a very potent catalyst. We are presently studying the effect of catalyst loading on the rate of the reactions and are planning to test the activity of this compound toward chiral organophosphorus agents.



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Small molecules that should mimic the activity of OPH in the hydrolysis of organophosphorus nerve agents have been synthesized. The modified synthesis and design of chemical catalysts for the destruction of CW Agents is being explored.

The catalytic properties of compound 1 in the hydrolysis of the methylparathion has been determined. At lower pH, a stoichiometric reaction is observed indicating neutralization of the catalyst. In order to provide further insights into this neutralization reaction, the hydrolysis reaction of methyl parathion in the presence of complex 1 in acetone-d_6/D_2O solution has been studied by ^{31}P NMR spectroscopy and identified a phenyl oxazoline ligand and a dimethylthiophosphate ligand. Final identification of this product was provided by an X-ray structural analysis, which indicated that 2 is a centrosymmetric dinuclear complexes in which two palladium phenyloxazoline complexes are linked by dimethylthiophosphate ligands . In the crystal, compound 2 is isolated in the form of a supramolecular aggregate with two molecules of *p*-nitrophenol that are hydrogen bonded to the dimethylthiophosphate ligands. The formation of 2 indicates racemization of the α-carbon of the serine residue. Such racemizations are not uncommon and have been previously observed.

Task 12: Development of Peptide and Antibody-Based Biosensors for the Detection of Biotoxins in Environmental and Medical Applications

Surface Plasmon Resonance allows early detection of blood-borne, signatory peptides from bacterial infections by influencing structural changes in surface-bound antibodies. (Collaborative project with Dr. Gale Wagner (College of Veterinary Medicine, Texas A&M University – College Station.) This project awaits additional funding resources.

Task 13: Detection of Bacterial Pathogens Using Bacteriophage Arrays and Biofluorescence.

1. We have established a system for isolation, characterization and rapid DNA sequencing of phages for a model pathogen, Burkholderia cepacea. The next step is to identify appropriate phages for detecting B. cepacea using a phage-based lysis assay based on fluorimetry. Phage can be used to detect and inactivate biowarfare agents of categories A, B, and C. This new project will adapt technologies developed for the use of enzymes for detection and detoxification of CW agents in order to utilize phage lytic enzymes to detect and decontaminate bacteriological BW agents. (Young)

2. Studies continue on the extracellular polysaccharide depolymerase associated with the phage Bcep781, a lytic phage of *Burkholderia cepacia*, our model bacterial pathogen which causes disease in both humans and plants. The phage carries this enzyme as a feature of its tail structure, so it can reach the cell surface of the bacterium, and it is specific for *B. cepacia*. Thus this enzyme is potentially capable of a diagnostic readout on the presence of this pathogen by causing the generation of reducing sugars from hydrolysis of the polysaccharide. Such an enzymatic diagnostic would be robust, field-implementable, and it does not require growth of the bacterium or the phage. We are taking two parallel approaches to characterizing this enzyme and evaluating its usefulness as a diagnostic. First, we have substantially purified the free enzyme by preparative isoelectric focusing and ion-exchange chromatography and demonstrated that its substrate is indeed EPS-I, one of the two polysaccharides produced by *B. cepacia*. In parallel, we have completed and analyzed the DNA sequence of Bcep781, and have identified a single gene, *orf43*, as the most likely candidate for the coding sequence for the depolymerase (Young)

Task 14: Development of Discriminative Organophosphate Neurotoxin Biosensing Systems Based on Multiple OPH Enzyme Monitors

Not initiated.

Task 15: Development of integrated microfluidic-based sensors for detection of chemical and biological weapons

A strategy for efficiently mixing solutions and carrying out multistep catalytic reactions in microfluidic systems has been developed. The approach involves immobilizing catalysts on microbeads, placing the beads into well-defined microreactor zones, and then passing reactants through one or more of

the reactor zones to yield products. The catalyst-modified beads effectively mix reactants and increase the effective surface area of the channel interior, both of which improve reaction velocities compared to open channels. This approach is demonstrated using two sequential reactions catalyzed by glucose oxidase and horseradish peroxidase. In addition to providing a general route to chemical synthesis within microfluidic systems, this design strategy may also be applicable to modeling reaction pathways within cells and to bio/chemical sensing applications.

A microfluidics-based sensing system that relies on electrochemical detection and electrogenerated chemiluminescent (ECL) reporting was described. The important result is that the ECL reporting reaction is chemically decoupled from the electrochemical sensing reaction. That is, the electrochemical sensing reaction does not participate directly in the ECL process, but because electrochemical cells require charge balance, the sensing and ECL reactions are electrically coupled. This provides a convenient and sensitive means for direct photonic readout of electrochemical reactions that do not directly participate in an ECL reaction and thus broadens the spectrum of redox compounds that can be detected by ECL.This approach can be implemented in either a two-electrode or bipolar (single electrode) configuration. By manipulating the placement and dimensions of the conductors, the photonic response can be enhanced. The system is used to electrochemically detect benzyl viologen present in solution and report its presence via $Ru(bpy)_3^{2+}$ (bpy=2,2'-bipyridine) luminescence.

We developed a microanalytical system for determining enzyme kinetics using a continuous-flow microfluidic system. This approach provides a new means for rapid determination of enzyme kinetics for clinical diagnostics, and drug discovery and screening. Additionally, it provides a means for rapidly identifying the presence of enzyme inhibitors, and thus could be integrated into a handheld platform for detecting biological weapons. The analysis is carried out by immobilizing the enzyme on microbeads, packing the microbeads into a chip-based microreactor (volume ~ 1.0 nL), and flowing the substrate over the packed bed. Data were analyzed using the Lilly-Hornby equation and compared to values obtained from conventional measurements based on the Michaelis-Menten equation. The two different enzyme-catalyzed reactions studied were chosen so that the substrate would be nonfluorescent and the product fluorescent. The first reaction involved the horseradish peroxidase-catalyzed reaction between hydrogen peroxide (H_2O_2) and N-acetyl-3,7-dihydroxyphenoxazine (amplex red) to yield fluorescent resorufin, and the second the β-galactosidase-catalyzed reaction of nonfluorescent resorufin-β-D-galactopyronoside to yield D-galactose and fluorescent resorufin. In both cases the microfluidics-based method yielded the same result obtained from the standard Michaelis-Menten treatment. The continuous flow method required about 10 μL of substrate solution and 10^9 enzyme molecules.

Key Research Accomplishments

The Texas A&M University System Digital EMS

- Development of PC104 based modem controllers and ICU
- Design of significantly smaller computer and communications packages.
- Design of personnel based wireless camera unit for disaster work

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

- Greatly expand the library of enzymes that can be used in a variety of applications
- Determined the capability for detoxifying enzymes with actual CW agents (USARMICD)
- Demonstrated ability of enzymes to be incorporated into robust decontaminating painted active surfaces (Reactive Surfaces, LTD) at NATO Trial in Cauzaux France
- Developed multi-component, enzyme-based optical biosensor immobilized on a fiber optic wave guide of a standard portable fluorescent detector
- A cotton towelette has been decorated with enzyme to produce a self-decontaminating material that is currently being tested for protection of animal models to CW agents
- Micro-contact printing of organic polymers has provided a matrix the permits the controlled distribution of biological cells prior to form bioarrays for metabolic screening
- Several different chemically-reactive surfaces have been constructed from polynuclear metal complexes which are being evaluated for the detection and destruction of CW agents
- A microfluidics-based sensor system that relies on electrochemical detection has been developed for the detection of chemical and biological agents

Reportable Outcomes

The Texas A&M University System Digital EMS

Presentations

DREAMS technology was presented at the following conferences/demos:

- Oct 2002 – Trinity Valley Conference in Liberty County, Texas
- Nov 2002 – Tele-Trauma Conference in Vermont
- Nov 2002 – Texas EMS conference in Austin, Texas
- 15 Nov 2002 – CBIRF Tech Day Demonstration at Naval Surface Warfare Center in Indian Head, MD
- 8 Jan 2003 – DREAMS Product Line Review, College Station, Texas
- 20 Feb 2003 – Liberty County Hospital presentation
- 14 May, 2003 - Demonstration for Future Combat Systems meeting held in College Station
- 18-20 June 2003 – HMMWV DREAMS variant was demonstrated to CBIRF on a training exercise at the Naval Surface Warfare Center in Indian Head, MD
- 22-25 September 22 - National EMS Conference, Las Vegas, NV

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

Publications

1. W. Zhan; R. M. Crooks "Microfluidic Logic Circuits" *J. Am. Chem. Soc.* **2003**, *125*, 9934-9935.
2. Y. Niu; L. Sun; R. M. Crooks "Determination of the Intrinsic Proton Binding Constants for Poly(amidoamine) Dendrimers via Potentiometric pH Titration" *Macromolecules* **2003**, *36*, 5725-5731.
3. G. P. Perez; W. G. Yelton; R. W. Cernosek; R. J. Simonson; R. M. Crooks "Gas Adsorption Gates Based on Ultrathin Composite Polymer Films" *Anal. Chem.* **2003**, *75*, 3625-3630.
4. G. H. Seong; J. Heo; R. M. Crooks "Measurement of Enzyme Kinetics using a Continuous-Flow Microfluidic System" *Anal. Chem.* **2003**, *75*, 3161-3167.
5. T. Ito; L. Sun; R. M. Crooks "Observation of DNA Transport through a Single Carbon Nanotube Channel Using Fluorescence Microscopy" *Chem. Comm.* **2003**, 1482-1483.
6. T. Ito; L. Sun; R. M. Crooks "Simultaneous Determination of the Size and Surface Charge of Individual Nanoparticles Using a Carbon Nanotube-Based

Coulter Counter" *Anal. Chem.* **2003**, *75*, 2399-2406. DOI: 10.1021/ac034072v.

7. R. W. J. Scott; A. K. Datye; R. M. Crooks "Bimetallic Palladium-Platinum Dendrimer-Encapsulated Catalysts" *J. Am. Chem. Soc.* **2003**, *125*, 3708-3709.
8. G. H. Seong; R. M. Crooks "Efficient Mixing and Reactions within Microfluidic Channels Using Microbead-Supported Catalysts" *J. Am. Chem. Soc.* **2002**, *124*, 13360-13361.
9. L. Sun; R. M. Crooks "Dendrimer-Mediated Immobilization of Catalytic Nanoparticles on Flat, Solid Supports" *Langmuir* **2002**, *18*, 8231-8236.
10. J. Alvarez; L. Sun; R. M. Crooks "Electroactive Composite Dendrimer Films Containing Thiophene-Terminated Poly(amidoamine) Dendrimers Crosslinked by Poly(3-Methylthiophene)" *Chem. Mater.* **2002**, *14*, 3995-4001.
11. L. Sun; R. M. Crooks "Interactions between Dendrimers and Charged Probe Molecules. 1. Theoretical Methods for Simulating Proton and Metal Ion Binding to Symmetrical Polydentate Ligands" *J. Phys. Chem. B.* **2002**, *106*, 5864-5872.
12. W. Li, Y. Li, C. M. Hill, K. T. Lum, and F. M. Raushel, "Enzymatic Synthesis of Chiral Organophosphothioates from Prochiral Precursors" *Journal of American Chemical Society* ***124***, 3498-3499 (2002).
13. F. M. Raushel, "Bacterial Detoxification of Organophosphate Nerve Agents" *Current Opinion in Microbiology* ***5***, 288-295 (2002).
14. Y. Li, S. D. Aubert, and F. M. Raushel, "Operational Control of Stereoselectivity During the Enzymatic Hydrolysis of Racemic Organophosphorus Compounds" *Journal of American Chemical Society* ***125,*** 7526-7527 (2003).
15. "Control of Mammalian Cell and Bacteria Adhesion on Substrates Micropatterned with Poly(ethylene glycol) Hydrogels", Won-Gun Koh,[1] Alexander Revzin,[2] Aleksandr Simonian,[3] Tony Reeves[3] and Michael Pishko*[1], *Biomedical Microdevices,* 2003, 5:1, 11-19.
16. Characterization of Oxidoreductase/ Redox Polymer Electrostatic Film Assembly by Surface Plasmon Resonance Spectroscopy, FTIR, and Ellipsometry on Gold". A.L. Simonian, A. Revzin, J. R. Wild, J. Elkind, and M. V. Pishko Anal.Chim.Acta, 2002, Vol. 466, 201-212.
17. "Mass Transfer in Amperometric Biosensors Based on Nanocomposite Thin Films of Redox Polymers and Oxidoreductases", Michael V. Pishko, Alexander Revzin, Aleksandr L. Simonian. *Sensors,* 2002, *2,* 79-90
18. "Glucose, Lactate, and Pyruvate Biosensor Arrays Based on Redox Polymer/Oxidoreductase Nanocomposite Thin Films Deposited on Photolithographically Patterned Gold Microelectrodes" Alexander F. Revzin, Kaushik Sirkar, Aleksandr Simonian, and Michael V. Pishko. Sensors and Actuators, B, 2002, Vol.81, pp.359-368.
19. Wang, I.-N., Deaton, J.D. and Young, R. (2003) Sizing the holin lesion with an endolysin-□-galactosidase fusion. J. Bacteriol. 185: 779-787.

20. Picot, F. P. Gabbaï "C_2-chiral dinucleating ligands with a 3,6-disubstituted pyridazine core" *Tetrahedron Lett.* **2002**, *43*, 11-13.
21. Mitsukimi Tsunoda, François P. Gabbaï "Hexacoordination of a dimethylsulfide molecule " *J. Am. Chem. Soc.* **2003**, *125*, 10492-10493. "Neurotoxicity induced in differentiated SK-N-SH-SY5Y human neuroblastoma cells by organophosphorus compounds." 2003. Hong, M.S., S.J. Hong, R. Barhoumi, R.C. Burghardt, K. C. Donnelly, J. R. Wild, V. Venkatraj, E. Tiffany-Castiglioni. Toxicol. Appl. Pharmacol., 186:110-118.
22. "Enzyme-based Biosensor for the Direct Detection of Fluorine-containing Organophosphates". 2002. A.L. Simonian, J.K. Grimsley, A.W. Flounders J. S. Schoeniger, Tu-Chen Cheng, J.J. DeFrank and J. R. Wild. Analytica Chimica Acta, 442:15-23.
23. "The Influence of External Environment Fluctuations on the Signal Formation of Microbiosensors". V B. Arakelian, 2002. J. R Wild, and A. L. Simonian. *Biosensors and Bioelectronics,* 16: 69-72.

Patents and Patent Applications

1. D. Albagli; L. Sun; R. M. Crooks "Photonic Signal Reporting of Electrochemical Events" CIP related to U. S. Patent Application Serial Number: 10/393,942. filed 2003.
2. W. Zhan; J. Alvarez; R. M. Crooks "Electrochemical Sensing in Microfluidic Systems using Electrogenerated Chemiluminescence as a Photonic Reporter of Electroactive Species" Filed 2003. U. S. Patent Application Serial Number: 10/393,942.

Industrial Interactions

1. Eclipse Sciences, Inc. was co-founded by Richard Crooks to commercialize technology developed under the DREAMS contract. Venture capital is currently being pursued in London, Cambridge, and Austin, Texas. Eclipse plans to develop a hand-held detector for detection of DNA and proteins associated with biological weapons of mass destruction. R. Crooks)

2. Collaboration with Reactive Surfaces, LTD, of Austin, Texas, has resulted in the development of enzyme-based decontaminating painted surfaces. The potential utility of the paint materials has been demonstrated in a NATO-sponsored trial with Soman which was held in Cazaux, France in September 2002. Those materials are being tested by DARPA in collaboration with Dugway Proving Grounds. (J. Wild and F. Raushel)

3. Collaboration with LynnTech, LTD, of College Station, Texas, has produced enzyme-based, self-decontaminating towelettes which are currently being evaluated on animal models designed to counteract dermal exposure. (J. Wild)

Related Funding Activities

The DREAMS project has resulted in a series of research extensions into additional fundamental research into the detection and decontamination of Chemical and Biological Warfare Agents. Those activities have been successful at competing for research grants at the NSF and NIH and other external support as indicated below. There are additional research activities that have been submitted as spin-offs pending external peer review.

"Self Assembled Nanostructures", P.I. Francois Gabbai, Center for Integrated Microchemical Systems – TAMU, 09/01/2002- 08/31/2003.

"Synthesis of Chiral Phosphorus Synthons." P.I. Frank Raushel, Texas Coordinating Board for Higher Education. 01.02 – 12.03.

"Enzymatic Detoxification of Chemical Warfare Agents". P.I. Frank Raushel, National Institutes of Health. 07/2003-06/2007.

"Development of Smart Biosensors for Monitoring of Chemical and Biological Toxins." PI - A. Simonian. 01/01/03 - 12/31/03. State of Alabama, Auburn University, Detection and Food Safety Center."

"Phage Binding for Continuous Anthrax Spore Detection", National Institutes of Health, 08.01.03 – 07.31.2005, PI V. Petrenko, Co-PIs - V. Vodyanoy, B. Chin, A. Simonian.

"Nanoparticle-based Biosensor for Direct Detection of Organophosphate Chemical Warfare Agents and Neurotoxic Pesticides". NSF, PI- Simonian, Co-PIs – J. Wild, and T. Good. 10/01/03 - 09/30/06).

Chemical Detectors for Chemical and Biological Agents. P.I. Richard Crooks. Sandia National Laboratory.

"Activated Organophosphorus Hydrolase for Coupling to Human Skin." Co-P.I. J. Wild) Department of Defense STTR Collaboration with LynnTech Inc., College Station, Texas. (06/2000 - 05/2004)

CONCLUSIONS

The Texas A&M University System Digital EMS

Efforts throughout the year on the civilian DREAMS vehicle have been focused on transitioning the current system from prototype development into a fielding unit for deployment in Liberty Co., TX. A set of communication tests was performed throughout the county to characterize and profile the communications coverage provided by the cell modem infrastructure that will be used for the initial deployment. Additionally, a new software development effort was begun to transition the current DREAMS patient information subsystem to accommodate new Liberty County data requirements.

Work was also focused on initial development, design, and implementation of the Deployable Telemedicine System (DTS). This system was built and demonstrated at the 2002 American Telemedicine Association meeting. The concept of the DTS is to move the functionality of the DREAMS vehicle and/or the DREAMS physician station into a rugged and transportable set of containers that can be shipped and deployed in remote areas in minimal time and with minimal effort.

Work was begun to design and build a military DREAMS vehicle based on the HMMWV platform. This system will accommodate some of the same functionality used within the civilian prototype with the additional functionality to support DoD use requirements for fielded ambulances.

The Texas A&M University System Detection and Remediation of Chemical and Biological Threat Agents Program

The interdisciplinary research teams in the Detection and Remediation of Chemical and Biological Threat Agents Program of the DREAMS project have developed productive, widely-based interactions involving academic, federal, and private company scientists, engineers, and educators through the Texas A&M University System. Postdoctoral fellows and doctoral students are receiving invaluable training as they are involved in the development of rapid detection systems for chemical and biological threat agents, contributing to and participating in first-responder emergency medical training, environmental detoxification, and equipment/personnel decontamination, as well as developing material for protecting America's food supply. The research has resulted in 23 peer-reviewed publications, numerous presentations, and invited lectures in 2002-2003. They have presented two patents and leveraged their research activities into several million dollars in competitive research grants from the NIH, NSF, and several other federal funding agencies, such as DOE

and DOD. The consortium is concerned about multiple threat agents which pose world-wide environmental, agricultural, and human health threats as the result of military or terrorist action, as well as natural biological blooms or accidental releases. The Dreams teams have established and extended collaborations with corresponding colleagues at the federal level, at NATO, and around the world. The third year of the project has extended their industrial ties and has even resulted in the development of a new company (Eclipse Science, Inc.) to develop hand-held detection devices for chemical and biological agents. The six various working teams range from environmental bioremediation of hazardous materials to deciphering fundamental mechanisms of action for important decontaminating enzymes and genes. The primary targets for the DREAMS project is the development of robust, discriminating chemical and biosensor detection systems as well as pursuing decontamination processes for chemical warfare agents. New efforts in expanding detector platforms from chemical to biological agents have been pursued in two of the laboratories this year, and studies with non-enzymatic chemically-active surfaces have been extended in two other groups. Several diverse, combinatorial libraries of decontaminating enzymes are being constructed to neutralize specific neurotoxins and develop enzyme-based biosensor systems that can discriminate between various types and species of neurotoxins.

One of the driving philosophies of the program involves the partnering with private companies, federal agencies, and academic colleagues through a series of grants, SBIR and STTR programs to develop future deployable systems which are expected to be useful for both military and civilian protection. These products and those developed from currently emerging discoveries will be integrated into the DREAMS Interact ambulance, an advanced emergency medical response vehicle that supports coordinated emergency communications, remote patient monitoring, and provides a platform for distributing and utilizing advanced chemical and biological systems for the detection, destruction, and decontamination of weapons of mass destruction.